Functional connectivity patterns of medial and lateral macaque frontal eye fields reveal distinct visuomotor networks

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Abstract

It has been previously shown that small and large amplitude saccades have different functions during vision in natural environments. Large saccades are associated with reaching movements towards objects, whereas small saccades facilitate the identification of more detailed object features necessary for successful grasping and manual manipulation. To determine whether these represent dichotomous processing streams, we used resting-state fMRI to examine the functional connectivity patterns of the medial and lateral FEF regions that encode large and small amplitude saccades, respectively. We found that the spontaneous BOLD signals of the medial FEF were functionally correlated with areas known to be involved in reaching movements and executive control processes, whereas lateral FEF was functionally correlated with cortical areas involved in object processing and in grasping, fixation, and manipulation of objects. The results provide strong evidence for two distinct visuomotor network systems in the primate brain that likely reflect the alternating phases of vision for action in natural environments.
Introduction

Saccades are rapid movements of the eyes that shift the line of sight to a new location in the visual field. Although saccades are remarkably stereotypical across a wide range of amplitudes (Bahill et al., 1975), studies of human gaze behavior in natural and dynamic environments have demonstrated that small and large amplitude saccades serve very different behavioral functions (Foerster et al. 2012; Hayhoe et al. 2003; Johansson et al. 2001; Land et al. 1999; Land 2009; Land and Hayhoe 2001). Large saccades, often accompanied by a head movement, are made to task-relevant objects in the environment and are followed by a reaching movement towards the object. In many cases, these large gaze movements are themselves preceded by an orienting movement of the trunk that is likely guided by memory (Land 2009). The initial large saccade towards the object is typically followed by a series of considerably smaller saccades on the object, which are thought to provide the necessary visual information about the object’s shape and texture for grasping and manipulation. In most cases, the eyes will then leave the object before manual manipulation has ended. It is presently unknown how the brain achieves the tight temporal relationship between large saccades and reaching movements, and between small saccades, grasps, and object processing. Here we tested the hypothesis that separate functional networks underlie these behavioral couplings. Prime candidates for testing this hypothesis are the frontal eye fields (FEF) that are located bilaterally in the anterior banks of the arcuate sulci in macaque monkeys (Bruce and Goldberg 1985; Bruce et al. 1985) and in the ventral branch of the superior precentral sulcus in humans (Amiez et al. 2006; Luna et al. 1998; Paus 1996). Although it is presently unknown how large and small saccades are encoded in human FEF, it is well
known that the macaque FEF contains a topographic map for contralateral saccadic eye movements with lateral FEF encoding small saccades and medial FEF encoding large saccades and head movements (Bruce et al. 1985; Elsley et al. 2007; Robinson and Fuchs 1969). Tracer studies support separate divisions of macaque FEF, showing that medial and lateral FEF differ in both their anatomical connectivity and cytoarchitecture (Schall et al. 1995; Stanton et al. 1995; 1993). Therefore, the known organization of macaque FEF and their widespread usage as a primate model for the neural control of saccades (Johnston and Everling 2008), reaching and grasping movements (Davare et al. 2011) makes these monkeys ideal subjects for investigating the neural networks associated with small and large saccades.

Resting-state fMRI (RS-fMRI) is quickly becoming the method of choice for investigating the organization of functional brain networks (Fox and Raichle 2007) across multiple species (Hutchison and Everling 2012) and their alterations across various psychiatric, neurological, and developmental disorders in the absence of experimental tasks (Greicius 2008; Menon 2011). Resting-state functional connectivity (FC) is largely constrained by the underlying anatomical architecture (Greicius et al. 2009; Honey et al. 2009; Shen et al. 2012; Vincent et al. 2007) and is presumed to be a hemodynamic manifestation of FC between slow fluctuations in neuronal activity (Scholvinck et al. 2010; Shmuel and Leopold 2008; for reviews see Fox and Raichle 2007; Leopold and Maier 2011).

Here, we used resting-state fMRI technique to directly determine the FC of medial and lateral FEF in macaque monkeys. We found that medial and lateral FEF form largely separate functional neural networks that may underlie the distinct behavioral roles of large and small saccades in the visual control of action.
**Materials and Methods**

**Subjects**

Five naive male macaque monkeys (three *Macaca mulatta* and two *Macaca fascicularis*), weighing between 5 and 8.5 kg, were the subjects in this study. These data have not been published in our previous reports (Hutchison et al. 2011a, 2011b, 2012a, 2012b; Shen et al. 2012) and functional data have been acquired at a higher spatial resolution (see below). All experimental methods were carried out in accordance with the Canadian Council of Animal Care policy on the use of laboratory animals and approved by the Animal Use Subcommittee of the University of Western Ontario Council on Animal Care.

**Data acquisition**

Data acquisition procedures in this study have been used previously to derive functional connectivity maps in previous studies (Hutchison et al. 2011a, 2011b, 2012a). On the day of scanning, the monkeys were anesthetized by intramuscular injections of atropine (0.4 mg/kg), ipratropium (0.025 mg/kg), and ketamine hydrochloride (7.5 mg/kg). Afterwards, 3 ml of propofol (10 mg/ml) was administered intravenously and oral intubation was carried out. Maintenance of anesthesia was conducted by using 1.5% isoflurane mixed with oxygen. Animals had spontaneous respiration throughout the duration of the experiment. The animals were placed in a custom-built chair designed for monkeys and they were head fixed while the monkeys were in the magnet bore. Isoflurane level was reduced to 1% during functional image acquisition. The animals’ vital signs were monitored throughout the duration of the image acquisition (rectal temperature via a fiber-optic temperature probe (FISO, Quebec City, QC), respiration via
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bellows (Siemens Corp., Union, NJ), and end-tidal CO2 via capnometer (Covidien-Nellcor, Boulder, CO). Physiological parameters were in the normal range throughout the image acquisition procedure (temperature: 36.8 °C; respiration: 24–32 breaths/min; end-tidal CO2: 31-40 mmHg). A heating disk (Snugglesafe, Littlehampton, West Sussex, UK) and thermal insulation were used to maintain body temperature. Anesthesia was used in this study because there are fewer motion artifacts, less physiological stress and no need to train the monkeys to stay in the scanner. Regardless of the vasodilator properties of isoflurane and its effects on cerebrovascular activity (Farber et al., 1997), network connectivity and synchronous BOLD fluctuations under isoflurane anesthesia have been robustly reported elsewhere (Hutchison et al. 2012a; Vincent et al. 2007). The data were acquired on an actively shielded 7 Tesla 68cm horizontal bore scanner with a DirectDrive console (Agilent, Santa Clara, CA) and a Siemens AC84 gradient subsystem (Erlangen, Germany) operating at a slew rate of 350 mT/m/s. An in-house designed and manufactured conformal 5-channel transceive primate head RF coil was used for acquiring magnetic resonance (MR) images. The coil consisted of an array of elements wrapped 270 degrees circumferentially around the head. Magnetic field optimization (Bo shimming) was performed using an automated, 3-dimensional mapping procedure over the specific imaging volume of interest. For each monkey, 10 runs of 150 continuous EPI functional volumes (TR = 2000 ms; TE = 16 ms; flip angle = 70°, matrix = 96 × 96; FOV = 96 × 96 mm; acquisition voxel size = 1 × 1 × 1 mm) were acquired, each scan totaling 5 min. EPI images were acquired with phase encoding in the left-right direction using GRAPPA at an acceleration factor of 2. Every image was corrected for physiological fluctuations using navigator-echo-correction. A high-resolution gradient echo (GRE) anatomical MR image was acquired along the same
orientation as the functional images \( (TR = 1100 \text{ ms}; \ TE = 8 \text{ ms}; \ \text{matrix}=256\times256; \)

\[ \text{FOV}=96\times96\text{mm}; \ \text{acquisition voxel size} = 375 \ \mu\text{m} \times 375 \ \mu\text{m} \times 1 \text{ mm}. \] Also, for every monkey

a T1-weighted anatomical image \( (\text{TE} = 2.5\text{ms}; \ TR = 2300\text{ms}; \ TI = 800\text{ms}; \ \text{FOV} = 96\times96\text{mm}; \)

\[ 750 \ \mu\text{m isotropic resolution} \) was acquired.

**Image preprocessing**

Functional MRI preprocessing was carried out using the FSL software package

(\url{http://www.fmrib.ox.ac.uk}). The preprocessing procedure included motion correction (six parameter affine transformation), brain extraction, spatial smoothing (FWHM = 2 mm), high-pass temporal filtering (Gaussian-weighted least-squares straight line fitting with sigma = 100 s),

low-pass temporal filtering (HWHM = 2.8 s, Gaussian filter), and non-linear registration (fMRIb non-linear image registration tool: FNIRT (\url{http://www.fmrib.ox.ac.uk/fsl/fslwiki/FNIRT})) to the individual monkey’s T2 weighted image. Global mean signal was not regressed out from the data because of its propensity for finding more anticorrelations (Murphy et al. 2009) and because it might remove physiologically important signals (Scholvinck et al. 2010).

**Statistical analysis**

For each monkey, spherical seed regions (radius of 2 mm) were selected individually in the medial and lateral FEF of the right and left hemisphere in the T2-weighted anatomical images (Fig. 1). There was no overlap between medial and lateral FEF seed regions and the distance between the two seeds were at least 4 mm. The mean time course of the signal for each seed region was extracted for each subject and each scanning session and was used as the regressor in
a generalized linear regression analysis. The regression model included the four seed time-series as predictors and also nuisance covariates (six motion parameters, white matter, and cerebrospinal fluid). Cardiac and respiratory activity was monitored, but not recorded during MR image acquisition and thus, white matter and cerebrospinal fluid (CSF) nuisance covariates were included in the model to further regress out the physiological noise from the data. This approach has been previously used to remove physiological noise in human and monkey resting-state fMRI studies (Hutchison et al. 2011a, 2011b, 2012a; Lund and Hanson 2001; Margulies et al. 2007; Shim et al. 2010). The cardiac rate is in the range of 1-2 Hz and thus, the cardiac signal can be aliased in the resting-state fMRI BOLD signal that has a lower frequency (0.01-0.1 Hz). There is no consensus in the literature about the best method of eliminating physiological noise from fMRI data (Churchill et al. 2012). Including the CSF and white matter nuisance covariates in our analysis is one of the preferable methods for physiological noise reduction, because it does not interfere with detection of functional activation (Shmueli et al. 2007). However, physiological noise can still affect our results (e.g. due to the different effects of cardiac and respiratory signal on CSF, white matter and grey matter), although we believe that the impact of physiological noise on the results is not substantial.

The first level of the analysis was carried out on the individual subjects at each scanning session. In the second level, the analysis results from the first level were normalized to the F99 atlas template (Van Essen 2004) and functional connectivity maps across ten scans for each monkey were generated by implementing a second-level fixed effects analysis using the FSL software package (http://www.fmrib.ox.ac.uk). The group level analysis was conducted by implementing a third-level fixed effect analysis. Corrections for multiple comparisons were
performed at the cluster level using Gaussian random field theory \((z > 5; \text{cluster significance: } p < 0.05, \text{corrected})\). It should be noted that the results obtained by fixed effects model cannot be extended to the population and thus, the results should be interpreted cautiously (Woolrich et al. 2004). A mixed effects analysis was not carried out due to the low subject number in our study. We also implemented another third level analysis in which we contrasted medial and lateral functional connectivity to determine whether the connectivity strength of an area is greater with medial or lateral FEF. All group level z-score maps were projected from volume data to the F99 cortical surface using the CARET (http://www.nitrc.org/projects/caret) enclosed voxel method (Van Essen et al. 2001). The F99 template is from a *Macaca mulatta*, but has also been successfully used as a template for *Macaca fascicularis* (Hutchison et al. 2011a, 2011b). A z-score threshold of 5 was used for the group maps to allow better visualization of the segregation between functionally connected areas. We overlaid a map of cortical subdivision by Van Essen et al. (2004) that has been previously mapped to both hemispheres of the F99 template (http://sumsdb.wustl.edu/sums/index.jsp).

**ROI identification**

In Table 1, we have calculated the percentage connectivity of parietal areas MIP, V6A, VIP and premotor areas F2, F4, F5 with medial and lateral FEF and we have provided corresponding statistics. The region of interest (ROI) of areas MIP and VIP are based on anatomical landmarks mentioned in Van Essen et al.’s atlas (Van Essen et al. 2012). The composite atlas of Van Essen et al. (2012) does not include area V6A and the ROIs corresponding to area V6A was defined based on the landmarks described by Galletti et al. (1999). Also, because premotor areas in most
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of the reaching/grasping literature are designated different than the nomenclature used by Van Essen et al. (2012), ROIs of premotor areas F2, F4 and F5 were selected based on boundaries defined in Markov et al.’s atlas (Markov et al. 2011).

Results

We placed spherical seeds in medial FEF regions corresponding to area 8Ac and portions of areas 8Am and 8As and in lateral FEF regions corresponding to area 6Vam and portions of area 45 (Van Essen et al. 2012) (Fig. 1). Figure 2 shows the FC of the right medial and lateral FEF superimposed on inflated ipsilateral and contralateral cortical surfaces (see Fig. 3 for seeds in medial and lateral FEF in the left hemisphere). For a more detailed comparison of the FC and previously identified cytoarchitectonic areas in the macaque, we superimposed the architectonical map by (Van Essen et al. 2012) on the flattened cortical surfaces of the left and right hemisphere. Red and green areas indicate regions that displayed FC with medial and lateral FEF, respectively. Areas in yellow indicate overlapping FC between lateral and medial FEF. These connectivity maps for the ipsilateral hemisphere indicate that areas in the intraparietal sulcus (IPS), lateral intraparietal area (LIP), parietal-occipital area (area PO) which has been subdivided into areas V6 and V6A (Galletti et al. 1996), posterior intraparietal area (PIP), medial dorsal parietal area (MDP), medial intraparietal area (MIP), area 7a, area 5D and dorsomedial areas in V1, V2, V3 are functionally connected to the medial FEF. Area PO has been subdivided into dorsal area V6A and ventral area V6 (Galletti et al. 1996) and we will use the V6A and V6 nomenclature in the rest of the paper because of its widespread use in reaching/grasping literature. In the right hemisphere, we found almost no FC of medial FEF with inferotemporal
areas, whereas some areas such as TE in the left hemisphere showed FC. Also, we found large
areas in the anterior bank of the superior temporal sulcus (STS), dorsal parts of middle temporal
area (MT) and medial superior temporal area (MST) that show positive FC with the medial FEF.
Furthermore, the connectivity maps show that medial FEF is functionally connected to the dorsal
somatosensory cortex (areas 1, 2, 3a, 3b), dorsal primary motor cortex (area 4), dorsal premotor
areas (6DR, 6Ds, 6DC or area F2 according to Markov et al.’s atlas (2011)) and supplementary
motor area (SMA). Premotor areas involved in reaching/grasping are most commonly referred to
as F2, F4 and F5 in the literature, e.g. see (Luppino et al. 1999). Area F2 includes areas 6DC,
6Ds and 6DR; area F4 encompasses ventral area 4C and caudal portions of areas 6Val and 6Vb;
and area F5 includes ventro-rostral area 6Val and rostral area 6Vb (Markov et al. 2011) as shown
in figures 4 and 5. We will use both terminologies in order to make it easier to compare our
results with existing literature. In the prefrontal cortex, several areas, including ventral area 46
along the principal sulcus, areas 9, 10, and 14 showed FC to the medial FEF. Interestingly, our
results show that the medial FEF has strong FC with medial wall of the ipsilateral hemisphere, i.e.
medial FEF is connected to parts of the anterior cingulate cortex (ACC) and posterior cingulate
cortex (areas 24 and 23, respectively). We also observed negative FC of the right medial FEF
with ventral premotor areas, ventral areas in central sulcus and portions area V4 are negatively
correlated with the medial FEF seed. In the contralateral hemisphere (Fig. 2A), we found
contralateral medial FEF functionally connected to the medial FEF seed. The connectivity
pattern of the medial FEF seed region in the contralateral hemisphere is very similar to that of
the same seed region in the ipsilateral hemisphere. However, the positively correlated areas in
the contralateral hemisphere are smaller in their spatial extent than the ipsilateral hemisphere.
Lateral FEF in the ipsilateral hemisphere is positively correlated with ventrolateral areas in the central sulcus, area 7t, anterior intraparietal area (AIP), area LIP and ventral intraparietal area (VIP). It should be noted that rostral LIP displays higher functional connectivity with lateral FEF than medial FEF. In the visual cortex, ventrolateral areas in V1, V2, V3 and a large portion of V4 and the ventral posterior area (VP) show functional connectivity with the lateral FEF. In the left hemisphere, we also found strong FC with inferotemporal areas, such as TE and ventral occipitotemporal area (VOT). In addition, ventral premotor areas exhibited functional connectivity with the lateral FEF. In the contralateral hemisphere, we observed almost same areas as in the ipsilateral hemisphere that are functionally connected to the lateral FEF seed. Negatively correlated areas with the lateral FEF seed were found within the cingulate cortex, lateral sulcus and small areas in the parahippocampal cortex.

Although the FC of medial and lateral FEF differed substantially, we also found overlapping FC between lateral and medial FEF (yellow areas in Fig. 4). Overlapping FC was present in portions of areas 4C and 6Ds corresponding to premotor area F4, caudal area LIP, lateral occipital parietal area (LOP), dorsal areas in the IPS such as ventral intraparietal area (VIP), area V3A and small areas in the MST. We have quantified the overlap in FC for several key parietal and premotor areas in Table 1. Little overlap was found for negatively correlated areas (Fig. 5).

Both the medial and lateral seeds were negatively correlated with the caudate nucleus and the rostral putamen (Fig.6. at 0-10 mm anterior to the anterior commissure). Positive correlations were found in the caudal putamen and globus pallidus (Fig. 6. at 5 and 10 mm posterior to the anterior commissure). There were also bilateral positive correlations of the medial FEF seed with
regions 20 mm posterior to the anterior commissure which might correspond to the superior colliculi, although they are located slightly too far dorsal for accurate identification.

Discussion

Behavioral studies have demonstrated that small and large saccades have different functions during vision in natural environments (Foerster et al. 2012; Hayhoe et al. 2003; Johansson et al. 2001; Land et al. 1999; Land 2009). Large saccades are associated with reaching and trunk movements towards objects, whereas small saccades likely promote the identification of more detailed object features for successful grasping and manual manipulation. Here we used resting-state fMRI in the macaque to test the hypothesis that this tight temporal coupling is reflected in the functional connectivity (FC) of medial and lateral FEF regions which encode large and small amplitude saccades (Bruce et al. 1985; Robinson and Fuchs 1969), respectively. We found that the spontaneous BOLD signal of areas known to be involved in reaching movements and executive control processes were functionally stronger connected with medial FEF than lateral FEF, whereas cortical areas involved in object processing and in grasping, fixation, and manipulation of objects showed stronger FC to lateral FEF than medial FEF. The results critically extend a previous RS-fMRI study that only placed a single seed region in the FEF (Hutchison et al. 2012a; Vincent et al., 2007) and previous traditional tracer studies (Schall et al. 1995; Stanton et al. 1995; 1993) and suggest that the FC of lateral and medial FEF reflect the different behavioral roles of small and large saccades in natural vision.

The limited visual acuity of the peripheral retina in primates necessitates gaze shifts that bring the high-resolution fovea onto regions of interest (Gilchrist 2012). Gaze shifts towards
targets of more than 20 degrees in amplitude also require a contribution of the head (Freedman and Sparks 1997). In many cases, gaze shifts do not just serve a visual search function, but provide the necessary location information for a subsequent reaching movement. Here we found that medial FEF is functionally correlated with parietal areas (e.g. PIP, MDP, MIP, 7a, V6A, 5D) and dorsal premotor areas (6DR, 6Ds, 6DC or area F2) that are known to be involved in reaching movements in macaques (Fattori et al. 2001; Grefkes and Fink 2005; Wise et al. 1997). We also found FC of medial FEF with cingulate areas (areas 23 and 24) and dorsal primary motor cortex, dorsal somatosensory cortex, and supplementary motor cortex that contain proximal and distal representations of the arm (Dum and Strick 2002; Strick et al. 1998). For visual areas, FC was found with V1, V2, V3, MT, and MST. This FC of medial FEF is consistent with the tight temporal coupling of large saccades and arm movements in eye-hand coordination (Angel et al. 1970; Biguer et al. 1982; Gribble et al. 2002; Prablanc et al. 1979). In addition, we observed strong FC of medial FEF with areas in prefrontal cortex (46, 9, 10, and 14) that are involved in executive control (Fuster 2008). While “free viewing” experiments have shown that salient visual features such as color, intensity, contrast, and sudden onsets influence the selection of saccade targets (Itti and Koch 2000), studies that have investigated gaze behavior under natural conditions have demonstrated that behavioral goals play a more dominant role in determining the distribution of gaze (Ballard and Hayhoe 2009; Tatler et al. 2011). Land has proposed that the dorsolateral prefrontal cortex is associated with setting and re-programming these behavioral goals that determine the location of gaze and reaching movements (Land 2009). In addition, we found FC of the medial FEF seed with areas in the posterior, medial, and anterior cingulate cortex that are known to have reward, performance, and outcome-monitoring activity (Amiez et
al. 2006; Emeric et al. 2008; Hayden et al. 2008; Ito et al. 2003; McCoy et al. 2003; Shima and Tanji 1998). Strong FC of medial FEF with these areas is consistent with the framework proposed by Tatler and colleagues in which gaze is allocated based on reward maximization (Tatler et al. 2011).

The FC of lateral FEF was consistent with the functional role of small saccades in vision and action in natural environments. In addition to early visual areas like V1, V2, and V3, lateral FEF was functionally correlated to V4 and areas like TEa and VOT in the caudal inferior temporal cortex that show colour and orientation-selectivity (Desimone and Schein 1987; Nakamura et al. 1994). We also found that lateral FEF had FC with parietal area AIP and ventral premotor area F5 and interestingly areas AIP and F5 are reciprocally connected (Rizzolatti and Luppino 2001). Area AIP is considered as a part of the lateral-dorsal stream (Galletti et al. 2003). This area contains neurons that code for specific kinds of grasping or manipulation movements and that have visual responses that may be encoding three-dimensional object characteristics (Murata et al. 2000; Sakata et al. 1995). A direct role of area AIP in grasping has been demonstrated by transient inactivation of the area in monkeys, which impairs the appropriate hand posture towards grasped objects (Gallese et al. 1994). Interestingly, fMRI studies have suggested that there might be an area homologous to macaque AIP in humans (Cavina-Pratesi et al. 2007; Culham et al. 2003).

As mentioned before, lateral FEF displayed functional connectivity with ventral premotor cortex (such as F5) that contains different classes of grasp-related neurons (Raos et al. 2006). Inactivation of area F5 in the posterior bank of the arcuate sulcus impairs the appropriate shaping of the hands during the grasping of objects (Fogassi et al. 2001). A direct role for ventral
premotor cortex in saccade control has been demonstrated by Fuji and colleagues who found a subregion on the gyral surface of the premotor cortex where saccades could be evoked by electrical microstimulation and where neurons were active during saccade tasks without arm movements (Fujii et al. 1998).

The concept of two parallel processing streams within the dorsal visual stream (Jeannerod et al. 1995) has been challenged and discussed recently (Fattori et al. 2010; Galletti et al. 2003; Rizzolatti and Matelli 2003; Mon-Williams and McIntosh 2000; Smeets and Brenner 1999). Although our results provide support for the existence of two sub-streams within the dorsal visual stream, there is a substantial overlap in FC between these two sub-streams (see Table 1). For instance, lateral FEF still exhibits weak FC with the medial parietal and dorsal premotor areas (Table 1). This finding has been supported by recent electrophysiological studies in monkeys. Fattori et al. (2010) have found grasping neurons in V6A and also area F2 has been shown to contain neurons with distal as well as proximal forelimb movement fields (Raos et al. 2003). Based on the results, it can be posited that FC of the lateral and medial FEF with reaching/grasping areas falls into a continuum; medial parietal areas are more strongly connected with medial rather than lateral FEF, whereas lateral parietal areas are highly connected to the lateral FEF. Area VIP that is located in between medial and lateral parietal areas is functionally connected to both medial and lateral FEF. The same argument can be made regarding the FC of FEF with dorsal and ventral premotor areas. Area F2 is functionally connected to medial FEF stronger than lateral FEF, area F5 displays higher FC to lateral FEF and area F4 is functionally connected to both FEF regions.

The results show that area LIP in the parietal cortex is also connected to both medial and
lateral FEF. Microstimulating the rostral area LIP triggers saccades in craniocentric rather than retinotopic coordinates (Kurylo and Skavenski 1991). Such a craniocentric coding has also been observed in the ventral premotor cortex (Fujii et al. 1998). Interestingly, we found that rostral area LIP was also functionally connected to the small saccade region of FEF, whereas we found that the more caudal region showed FC with both medial and lateral FEF seeds.

Within subcortical areas, we observed significant positive FC between the caudal putamen and FEF. Interestingly, there is evidence from anatomical tracing studies that FEF projects to the caudal putamen in monkeys (Parthasarathy et al. 1992; Stanton et al. 1988). These projections might have a role in saccade control as well as in hand movements. As a support to this notion, saccade-related and saccade outcome-related activity has been recently reported in the caudal macaque putamen (Phillips and Everling, 2012) and Ueda and Kimura (2003) have proposed that the putamen may be involved in visuospatial processing of hand movements. We also observed that both medial and lateral FEF have negative FC with the caudate nucleus and the rostral putamen. Although still poorly understood, it has been suggested that spontaneous anti-correlated activity at rest exists between systems with oppositely signed responses during task performance (Fox et al. 2009). However, previous studies have demonstrated that the caudate nucleus facilitates saccades (Hikosaka et al. 2000), whereas we found that the caudate had negative correlation with FEF. Further studies are needed to reveal the neural mechanisms underlying anti-correlated resting-state fMRI activity.

FC as identified by RS-fMRI is based on the correlations of spontaneous low-frequency fluctuations of the BOLD fMRI signals between different brain areas and as such does not necessarily indicate that functionally connected brain areas are also anatomically linked by
monosynaptic connections. However, systematic studies that compare RS-fMRI FC to a large set of data from tracer studies in macaques have shown that FC patterns in RS-fMRI are dictated by the underlying neuroanatomical architecture (Adachi et al. 2012; Shen et al. 2012). This observation is consistent with an earlier RS-fMRI study that had placed a single seed in FEF and reported positive FC with known anatomically connected areas in the posterior and frontal cortex (Hutchison et al. 2012a) and strong anti-correlated FC with areas in the lateral sulcus.

Resting-state FC maps could be affected by a variety of factors. For instance, if the physiological noise originating from cardiac and respiratory activity is not removed from the data, it would substantially affect the FC maps. Moreover, anesthesia per se can alter resting-state FC; for instance it has been shown that the FC of posterior cingulate cortex in the default mode network was decreased under propofol (Boveroux et al. 2010) and sevoflurane (Martuzzi et al. 2010) administration. Moreover, activity in executive control networks has been shown to be decreased under anesthesia (Boveroux et al. 2010). Animal studies have also shown that resting-state FC changes under anesthesia and it has been posited that different levels of anesthesia change the specificity of FC maps (Liu et al. 2012). However, the same study has shown that the BOLD signal correlation is strongest between bilateral homotopic brain regions, even under deep anesthesia (Liu et al. 2012). Vanhaudenhuyse et al. (2010) have suggested that reductions in FC could be interpreted as reduced capacity for cognitive processing. The alternative explanation could be that although anesthesia can alter the resting-state FC, the physiologic ”baseline” FC still persists regardless of the altered level of consciousness. This idea has been supported in previous resting-state fMRI studies (e.g. Raichle et al. 2001). Similarly, Vincent et al. (2007) have shown that the coherent spontaneous BOLD fluctuations in the
monkey oculomotor system persist during light and deep anesthesia. Furthermore, resting-state FC that has been acquired in anesthetized monkeys corresponds well with the anatomical connectivity between brain areas as mentioned above (Hutchison et al. 2012a; Shen et al. 2012). In the present study, we have acquired the functional images under 1% isoflurane anesthesia that is defined as light anesthesia (Vincent et al., 2007), minimizing the effects on resting-state FC.

The distinct FC pattern of medial and lateral FEF with other cortical areas is in good agreement with the results from anatomical tracer studies. A retrograde tracer study reported that lateral FEF projected to V3, V4, V4T, TE, FST, MT, MST, and rostral LIP, whereas medial FEF innervated V2, VIP, 23b, MDP, DP, 7a, and caudal LIP (Stanton et al. 1995). A similar anatomical connectivity pattern has been described for projections from posterior areas to FEF (Schall et al. 1995). Overall, that study demonstrated that medial FEF receives projections predominately from posterior regions representing the peripheral visual field whereas lateral FEF receives inputs from areas that encode the central visual field (Schall et al. 1995). The FC patterns are also similar to the anatomical connectivity of FEF with frontal regions (Stanton et al. 1993), with the exception of the supplementary eye fields (6DR) which receive projections from medial and lateral FEF, but which only showed FC with medial FEF. Functionally connected areas in the hemisphere contralateral to the seed ROIs were smaller in spatial extent compared to functionally connected areas in the ipsilateral hemisphere. Although it is possible that physiological noise may contribute to this finding, it is more likely that the finding has a neural origin. Anatomical tracing studies have posited that connectivity in the contralateral hemisphere is commonly restricted to geographically and architectonically homotopic areas of the cortex (e.g. see Lewis and Van Essen, 2000). Thus, the FC with contralateral heterotopic areas are likely
polysynaptic and this may explain the smaller functionally connected areas in the contralateral hemisphere.

The medial FEF network contained areas of the medio-dorsal stream and the lateral FEF network included areas in the lateral-dorsal processing stream as well as areas in the ventral visual stream. Therefore, we believe that the organization of these two networks reflects the alternating phases of vision for action in natural environments: A re-orienting phase that is usually based on behavioral goals which involves the generation of a large saccade or eye-head movement together with a reaching movement of the arm and a subsequent detailed object processing phase that involves small saccades, grasping, and manual manipulation.

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Functional connectivity of medial and lateral FEF


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**Figure legends**

Fig. 1. The location of lateral and medial FEF spherical seeds in Monkey 1. The spherical seed regions have a radius of 2 mm.

Fig. 2. Right medial (left) and right lateral (right) FEF seed connectivity maps projected on the F99 template (Van Essen, 2004). The connectivity maps are shown on lateral, medial, dorsal and ventral views. The asterisks show the location of the seed region. pos, parietooccipital sulcus; cas, calcarine sulcus; cs, central sulcus; hs, hippocampal sulcus; cis, cingulate sulcus; sts, superior temporal sulcus; ios, inferior occipital sulcus; lus, lunate sulcus; ots, occipitotemporal sulcus; ps, principal sulcus. (z-score > 5 set at cluster significance of P < 0.05, corrected for multiple comparisons).

Fig. 3. Left medial (left) and right lateral (right) FEF seed connectivity maps projected on the F99 template (Van Essen, 2004). The connectivity maps are shown on lateral, medial, dorsal and ventral views. The asterisks show the location of the seed region. pos, parietooccipital sulcus; cas, calcarine sulcus; cs, central sulcus; hs, hippocampal sulcus; cis, cingulate sulcus; sts, superior temporal sulcus; ios, inferior occipital sulcus; lus, lunate sulcus; ots, occipitotemporal sulcus; ps, principal sulcus. (z-score > 5 set at cluster significance of P < 0.05, corrected for multiple comparisons).
temporal sulcus; ios, inferior occipital sulcus; lus, lunate sulcus; ots, occipitotemporal sulcus; ps, principal sulcus. (z-score > 5 set at cluster significance of $P < 0.05$, corrected for multiple comparisons).

Fig. 4. Flattened cortical views of both hemispheres to display spatial overlap connectivity patterns of right medial and lateral FEF (A) and left medial and lateral FEF (B). The figures show the connectivity pattern of the positively functionally connected areas (z-score > 5 set at cluster significance of $P < 0.05$, corrected for multiple comparisons). Areas in red are functionally connected to the medial FEF, areas in green are functionally connected to the lateral FEF and areas in yellow are functionally connected to both. The white lines indicate the boundaries of parietal areas V6 and V6A according to Galletti et al. (1999) and also premotor areas F2, F4 and F5 according to Markov et al. (2011). The asterisks show the location of the seed regions.

Fig. 5. Flattened cortical views of both hemispheres to display spatial overlap connectivity patterns of right medial and lateral FEF (A) and left medial and lateral FEF (B). The figures show the connectivity pattern of the negatively functionally connected areas (z-score > 5 set at cluster significance of $P < 0.05$, corrected for multiple comparisons). Areas in red are negatively connected to the medial FEF, areas in green are negatively connected to the lateral FEF and areas in yellow are negatively connected to both. The white lines indicate the boundaries of parietal areas V6 and V6A according to Galletti et al. (1999) and also premotor areas F2, F4 and F5 according to Markov et al. (2011). The asterisks show the location of the seed regions.
Fig. 6. Coronal slices of the functional connectivity patterns of medial and lateral FEF on F99 atlas (Van Essen, 2004) as indicated at the top of each column. The red-yellow areas are positively and blue-light blue areas are negatively correlated areas (z-score > 4 set at cluster significance of P < 0.05, corrected for multiple comparisons).
Table 1.
Statistical analysis and percentage connectivity of FEF seed regions with parietal and premotor areas

<table>
<thead>
<tr>
<th></th>
<th>Posterior Parietal Cortex</th>
<th>Premotor Cortex</th>
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<tbody>
<tr>
<td></td>
<td>MIP</td>
<td>V6A</td>
</tr>
<tr>
<td>Medial FEF &gt; Lateral FEF (z-score)</td>
<td>3.5*</td>
<td>1.96</td>
</tr>
<tr>
<td>Lateral FEF &gt; Medial FEF (z-score)</td>
<td>0.02</td>
<td>0.22</td>
</tr>
<tr>
<td>% Connectivity to medial FEF seed</td>
<td>74.66</td>
<td>71</td>
</tr>
<tr>
<td>% Connectivity to lateral FEF seed</td>
<td>25.34</td>
<td>29</td>
</tr>
</tbody>
</table>

Note: *: statistically significant. % Connectivity to medial FEF seed = \( \frac{Z_m}{Z_m + Z_l} \) * 100. % Connectivity to lateral FEF seed = \( \frac{Z_l}{Z_m + Z_l} \) * 100. \( Z_m \): Mean connectivity z-score to medial FEF seed. \( Z_l \): Mean connectivity z-score to lateral FEF seed. 